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## A selective decrease in the number of GABAergic somata occurs in pre-seizing monkeys with alumina gel granuloma

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Previous studies have shown that a loss of GABAergic neuronal somata is associated with a loss of GABAergic terminals at chronic cortical epileptic foci in monkeys. The present study was undertaken to determine whether GABAergic neuronal loss occurs prior to the onset of clinical seizures in monkeys that were treated with alumina gel but did not display seizures. Seven adolescent (*Macaca mulatta*) monkeys received alumina gel implants into the left pre- and post-central gyri, specifically centered in hand-face regions of sensorimotor cortex. Three other monkeys were used as controls. Two of these were surgical controls and the third was a normal animal. Three monkeys (pre-seizing) were sacrificed 2–4 weeks after the alumina gel implant but prior to clinically active seizures. Three other monkeys with chronic seizure activity (chronically seizing) were sacrificed 3–6 months after the implant. Tissue sections were taken from an area adjacent to the alumina gel granuloma (focus), from a site distal to it (parafocus) and from the non-epileptic contralateral side. Sections from all monkeys were processed for glutamate decarboxylase (GAD) immunocytochemistry and then examined with a light microscope. In addition, adjacent sections were stained with a Nissl stain and the total number of neurons was counted in these sections. Statistical analysis showed a significant decrease in the number of GAD-positive cells in the pre-seizing and chronic animals. The pre-seizing monkeys showed a significant loss of 23–44% at the focus in contrast to the total number of neurons which did not change significantly. The loss of GAD-positive cells was greater in the chronic animals that showed significant losses at both the focus and parafocus, 42–61% and 15–26%, respectively. It is important to note that the chronic monkeys displayed an 11–61% significant loss of total neurons at the epileptic focus. The surgical control animals showed no seizure activity and no significant loss of total neurons or GAD-positive cells. The main finding of this study indicates that a selective loss of GAD-positive neuronal somata occurs in pre-seizing monkeys with alumina gel implants. This finding is consistent with the previously reported loss of GABAergic terminals in pre-seizing monkeys. Since virtually all monkeys treated with alumina gel develop seizures, the results of this study add further support to the hypothesis that GABA neuronal loss plays a causal role in focal epilepsy.

### INTRODUCTION

Experimental models of focal epilepsy have been examined for specific cellular and biochemi-

cal changes to obtain an understanding of certain types of human epilepsy<sup>1,2,4,10,13,21,24</sup>. The alumina gel model in monkeys is an excellent model of post-traumatic epilepsy in humans<sup>13</sup>. Also, this model best approximates the histology of human foci in that they are both characterized by neuronal loss, reactive gliosis, and dendritic abnormalities<sup>3,5,15,18,25,26</sup>. Seizures usually begin 2–3 months

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following the alumina gel implants into the hand region of the sensorimotor cortex. This model has been analyzed with biochemical, anatomical, physiological, and pharmacological methods. Epileptic monkeys with alumina gel implants display spike and wave activity on electroencephalograms (EEGs) and electrocorticograms (ECoGs) which arises from the site adjacent to the alumina granuloma and a surrounding area of cortex<sup>27</sup>. Excision of these two regions abolishes epileptic activity<sup>6</sup>. In addition, extracellular recordings from cells in the focus display abnormal excitatory post-synaptic potentials<sup>27</sup>.

A growing body of evidence indicates that neurons containing the neurotransmitter gamma-aminobutyric acid (GABA) are preferentially lost in the epileptic foci of monkeys treated with alumina gel. Binding studies of these monkeys have indicated a decrease in the number of GABA receptors and biochemical studies have demonstrated a loss of tissue GAD activity in the focus<sup>1</sup>. Consistent with these findings were the initial immunocytochemical data that demonstrated a 58–62% loss of GABAergic axon terminals in the epileptic focus of chronically seizing monkeys that had not reached status epilepticus<sup>19</sup>. Subsequent studies have indicated that greater than 80% of the axon terminals of GABAergic chandelier and basket cells have degenerated at epileptic foci<sup>17,18</sup>. More recent immunocytochemical studies have shown that the loss of these terminals at chronic epileptic foci was due to a loss of GABAergic somata<sup>20</sup>. However, it was not shown whether a significant loss of GABAergic somata precedes the onset of seizure activity because only one pre-seizing monkey was used in that study<sup>20</sup>. This question needs to be resolved because recent findings have shown a loss of GABAergic terminals prior to the onset of clinical seizures<sup>8</sup>. Therefore, the present study of alumina gel-treated monkeys was undertaken using a larger number of pre-seizing monkeys to determine whether a significant loss of GABAergic somata occurs prior to the onset of seizure activity. Additional monkeys with chronic epilepsy were also studied. Finally, Nissl-stained sections from all monkeys were analyzed to determine the effect of alumina gel-induced seizures on the total number of cortical neurons. These latter data

would determine whether the loss of GABAergic somata was selective.

## MATERIALS AND METHODS

Ten adolescent monkeys (*Macaca mulatta*) were used in this study. Two of the monkeys (RB 160 and 161) were used in a previous study<sup>20</sup>. The monkeys weighed from 3 to 5 kg, were neurologically normal, and had normal initial scalp EEGs. The animals were housed in a controlled environment, in individual cages, under the care and supervision of veterinarians who specialize in the care of non-human primates.

Under general anesthesia, 9 of the monkeys underwent a left frontoparietal craniotomy. The precentral (Brodmann's area 4) motor cortex for the hand-face area was identified by electrocortical stimulation. Seven monkeys (RB-160, 161, 175, 176, 180, 184, 185) were injected with 0.2 ml of aluminum hydroxide (alumina gel) into the left pre- and post-central gyri using the Ward modification of the Kopeloff technique<sup>12</sup>. Two monkeys (RB-182 and RB-183) were used as surgical controls in which a subpial resection was made of a 5 × 5 mm area down to the white matter. This area is approximately equivalent to the area of a mature alumina granuloma. One monkey (RB-179) received neither an injection of alumina gel nor a surgical lesion and served as a control animal.

Postoperatively none of the animals received anticonvulsants. All animals were clinically observed on a daily basis for seizures. In addition, weekly scalp EEGs were performed postoperatively on all monkeys until the time of sacrifice except for 2 monkeys (RB-184 and RB-185) that had subdural electrodes placed for ECoG monitoring on a weekly basis. The electrophysiological changes, in large part, determined the time when anatomical changes were investigated. Chronic seizure monitoring was similar to that previously recorded<sup>1</sup>.

Three monkeys (RB-161, 175, 176) were sacrificed 2–4 weeks post-injection time which was prior to the development of seizure activity (Table I). These animals are referred to as pre-seizing monkeys and, although they did not display seizures, they demonstrated a sharp wave activity adjacent to the granuloma on the ECoG. The acute

seizing monkey (RB-160) was sacrificed 6 weeks following the injection. This animal displayed 2–3 seizures/day for 4 days. The fifth monkey (RB-179) was a normal control animal which displayed a normal ECoG. The sixth monkey (RB-180) displayed 3 seizures/week and was sacrificed 3 months following the alumina gel injection. The seventh monkey (RB-184) was sacrificed 5 months post-injection time and displayed 1 seizure/week. The last of the chronically seizing monkeys (RB-185) exhibited 1 seizure/week and was sacrificed 6.5 months following the injection. These chronically seizing animals showed an ECoG consistent with spike and wave discharges in the area immediately adjacent to the developing granuloma. Two other animals (RB-182, 183) that were surgical controls were sacrificed 2.5 months after the surgical lesion procedure and demonstrated no ECoG abnormalities.

After the ECoG, each monkey was anesthetized with sodium phenobarbital and perfused transcardially with approximately 2 liters of a 0.2% paraformaldehyde solution so that receptor studies could be made on the same brains. The skull and dura were removed after the perfusion. A tissue block from the left precentral gyrus was immediately removed and included an area adjacent to the alumina gel injection and an area 1–2 cm superior to the injection site. A block of tissue was also taken from the right precentral gyrus to be used as control tissue. These blocks were placed in a 10% formalin solution for further fixation. The tissue was then placed in a cryoprotectant solution of 30% sucrose in phosphate-buffered saline (PBS) for 24 h. Sections of 40  $\mu$ m thickness were cut on a freezing microtome and placed in 0.1 M PBS for light microscopic immunocytochemistry. Free floating sections were processed for the immunocytochemical localization of glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA, using an anti-GAD serum developed by Oertel et al.<sup>14</sup>. Sections from the right and left hemispheres were incubated at the same time. A modification of Oertel's protocol was used employing the avidin–biotin–horseradish peroxidase complex (Vector Labs.)<sup>9</sup>. Briefly, the sections are incubated in 0.1 M D,L-lysine in 10% normal rabbit serum for 12 h and then incubated in the primary

TABLE I

*Survival time vs. seizure activity of 10 monkeys (Macaca mulatta)*

<i>Animal</i>	<i>Alumina gel</i>	<i>Survival time*</i>	<i>Seizure activity**</i>
RB-161 pre-seizing	+	1	–
RB-175 pre-seizing	+	1	–
RB-176 pre-seizing	+	0.5	–
RB-160 acute	+	1.5	+
RB-180 chronic	+	3.0	+++
RB-184 chronic	+	5.0	+
RB-185 chronic	+	6.5	+
RB-179 normal	–	–	–
RB-182 sham***	–	2.5	–
RB-183 sham***	–	2.5	–

\* Survival time in months.

\*\* + equals 1 seizure/week.

\*\*\* Surgical controls.

antiserum raised in sheep against GAD for 24–28 h. Following three 10 min rinses in Tris-buffered saline (TBS), the sections are incubated in the biotinylated secondary antibody, rabbit antisheep IgG (1:227) for 45 min. After 3 more 10 min rinses in TBS, the sections are then incubated for 45 min in a mixture of avidin and biotinylated peroxidase complex (1:114). Following 3 additional rinses in TBS, the sections are reincubated with the secondary antibody for 30 min, rinsed again in TBS and reincubated in the avidin biotinylated peroxidase complex for 30 min. After another 3 rinses in TBS, the sections are reacted for 15–30 min in a DAB solution (6 mg diaminobenzidine/10 ml TBS + 0.002% hydrogen peroxide). Following three 10 min rinses in TBS, the sections were then mounted on subbed glass slides and air dried. These sections were then defatted in 50% chloroform and placed in alternating solutions of 0.0005% osmium tetroxide and 0.05% of thiocarbonylhydrazide to intensify the reaction product. The tissue was then dehydrated and coverslipped.

Sections of tissue were examined under a light microscope equipped with a 40  $\times$  objective lens. All sections were analyzed for the content of GAD-positive cells. The GAD-positive cells were identified as cells with dense, brownish reaction product within their perikaryal cytoplasm. The alumina gel-treated animals (Table I) were exam-

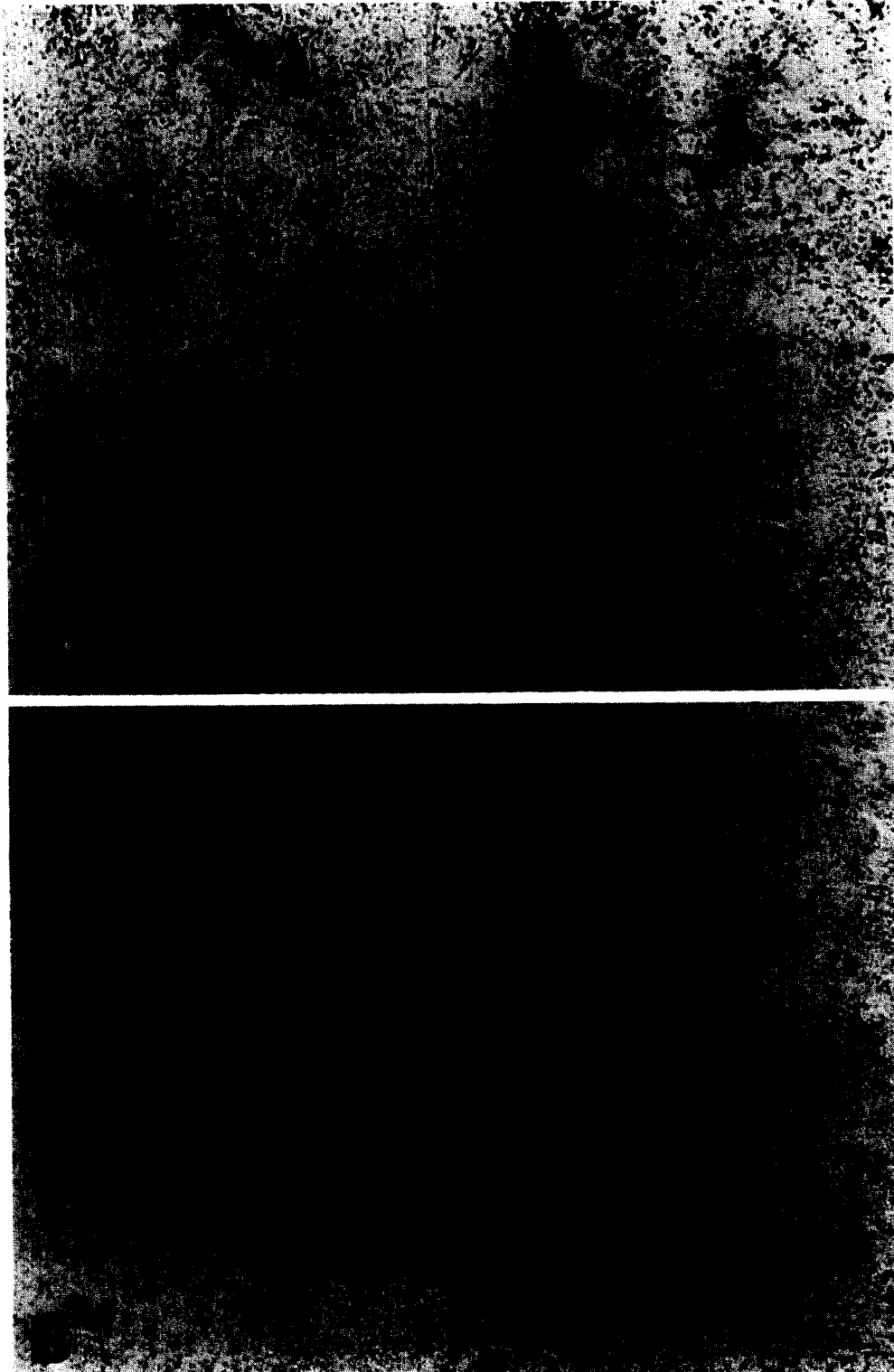


Fig. 1. Light photomicrographs of GAD-immunoreacted sections from a pre-seizing alumina gel-treated monkey (RB-161). A shows the parafocus where a number of GAD-positive cells (arrows) are found in layer V of motor cortex. Two large pyramidal cells that are non-immunoreactive are apposed by numerous GAD-positive puncta (arrowheads). B shows the focus from this same monkey. Note that there are less GAD-positive cells (arrows) as well as puncta (arrowheads) adjacent to pyramidal cells as compared to the parafocus. The contralateral non-epileptic site (not shown) was similar to the parafocus. Bar = 25  $\mu$ m.

ined and counted for GAD-positive cells at 4 sites. The area adjacent to the alumina granuloma was referred to as the focus, whereas an area 1 cm from the focus was called the parafocus. Two other sites were examined for these monkeys, and they were located in the contralateral hemisphere. The 2 surgical control animals were examined at a proximal site adjacent to the lesion, at a distal site about 1 cm away from the lesion, and at 2 other sites on the contralateral side. The normal monkey was counted for the number of GAD-positive cells at 2 sites 1 cm apart in both right and left motor cortices. A grid reticule that covered a space which was 282  $\mu\text{m}$  wide was used to count the number of GAD-positive somata. Three adjacent traverses were made through the entire thickness of the cortex. In addition, other sections through the motor cortex were stained with Cresyl violet, a Nissl stain. They were used to assess the total number of neurons at each site for every monkey by counting those neurons with nuclei in the section. Neuronal nuclei were distinguished from those of neuroglial cells by their sizes and morphologies.

The 10 animals were examined by statistical variance testing to determine if there were differences among the number of somata counted (Tables II and III). These animals were separated into 5 groups classified as normal, surgical control, pre-seizing, acutely seizing, and chronically seizing. Each group contained 4 subgroups that were also compared. The pre-seizing, acute, and chronically seizing animals had 4 subgroups called the focus, parafocus, and contralateral one and contralateral two sites (Table II). The normal animal had 2 subgroups on the ipsilateral side and 2 on the contralateral side. The surgical control animals had proximal, distal, and 2 contralateral subgroups. Then, a 1-way analysis of variance (ANOVA) was used to determine if there were any significant differences among the interactions between groups for each of two sites (i.e., focus and parafocus). Post hoc testing was used to determine specific between-group differences.

## RESULTS

### *Qualitative description*

All layers of the normal and non-epileptic motor

TABLE II

*Results of GAD-positive cell counts from 10 monkeys (number of somata/0.795 mm<sup>2</sup>)*

<i>RB-179 (normal)</i>		<i>RB-176 (pre-seizing)</i>	
1st site	20.5	Focus	21.9
2nd site	20.5	Parafocus	28.7
Cont. *	23.3	Cont.	38.3
Cont.	24.6	Cont.	39.7
<i>RB-182 (surgical control)</i>		<i>RB-160 (acute)</i>	
Proximal to lesion	19.2	Focus	13.7
Distal to lesion	21.9	Parafocus	19.1
Cont.	20.5	Cont.	16.4
Cont.	19.2	Cont.	17.8
<i>RB-183 (surgical control)</i>		<i>RB-180 (chronic)</i>	
Proximal to lesion	10.9	Focus	15.0
Distal to lesion	12.3	Parafocus	26.0
Cont.	13.7	Cont.	35.6
Cont.	15.0	Cont.	34.2
<i>RB-161 (pre-seizing)</i>		<i>RB-184 (chronic)</i>	
Focus	16.4	Focus	13.7
Parafocus	21.9	Parafocus	27.4
Cont.	20.5	Cont.	34.2
Cont.	21.9	Cont.	35.6
<i>RB-175 (pre-seizing)</i>		<i>RB-185 (chronic)</i>	
Focus	17.8	Focus	28.7
Parafocus	24.6	Parafocus	42.4
Cont.	27.4	Cont.	49.2
Cont.	26.0	Cont.	50.6

\* Cont. indicates the contralateral cortex.

cortex contained GAD-positive cells that were, in general, homogeneously distributed throughout all layers. However, somewhat fewer immunoreactive neurons occurred in layer I and the deeper part of layer VI of the motor cortex as compared to the other layers. The GAD-positive cells contained a brown reaction product in their perikaryal cytoplasm and rarely were their dendrites stained. Many of these neurons displayed eccentrically placed nuclei. The shapes of the cell bodies were clearly non-pyramidal (Figs. 1 and 2). Thus, the GAD-positive somata were round, oval, teardrop shaped, or fusiform shaped.

The sizes of GAD-positive cells varied as similarly shown in previous studies<sup>7,20</sup>. Most of the somata in layer I were small whereas somata in the deeper layers displayed a wide variety of sizes.

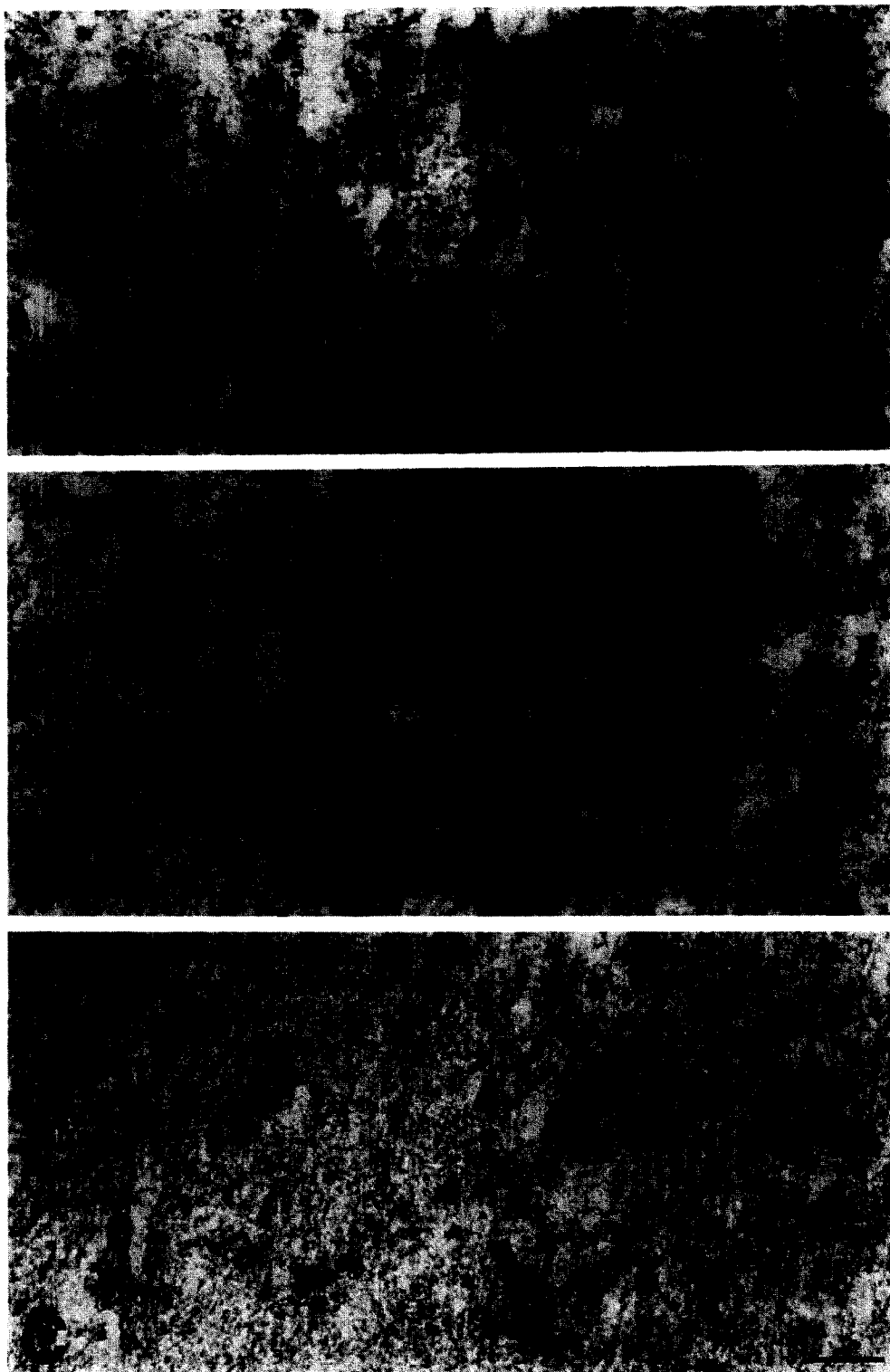


Fig. 2. Light photomicrographs of GAD-immunoreacted sections from a chronically seizing, alumina gel-treated monkey (RB-184). A shows layer V in the contralateral non-epileptic site that contains a number of densely stained GAD-positive cells (arrows) and numerous GAD-positive puncta (arrowheads). Some of the puncta are concentrated around a pyramidal cell. B is taken from layer V in the parafocus, a site 1 cm from the focus. The number of GAD-positive cells (arrow) and puncta (arrowheads) is less than that found in A. C shows the distribution of GAD-positive cells (arrow) in layer V of the focus adjacent to the alumina gel granuloma. Some GAD-positive puncta (arrowheads) are found adjacent to a non-immunoreactive pyramidal cell. Bar = 25  $\mu$ m.

Small cells were found throughout all layers of the cortex. However, the average somal area for GAD-positive cells increased from the superficial to the deep laminae.

GAD-positive somata that were found in the focus and parafocus sites were similar in shape and size to those in the normal and non-epileptic cortical sites (Figs. 1 and 2). Also, they were distributed throughout the cortical layers. However, reduced numbers of GAD-positive somata occurred in these regions of chronically seizing monkeys as compared to the 2 sites examined in the contralateral cortex (Fig. 2B and C). The pre-seizing monkeys displayed reduced numbers of GABAergic neurons in the focus (Fig. 1B). The parafocus in these monkeys appeared similar to the contralateral non-epileptic cortex. In addition to these observations for GAD-positive somata, the focus in both pre-seizing and chronically seizing monkeys displayed a reduction in the number of GAD-positive puncta as compared to the contralateral cortex (Figs. 1B and 2C).

The Nissl preparations of normal and non-epileptic cortices revealed the typical lamination patterns of neurons in the motor cortex (Fig. 3B). A prominent feature of this region, the large pyramidal cell of Betz, was found in layer V (Fig. 4). The Nissl preparations of pre-seizing motor cortex adjacent to the alumina gel granuloma displayed the same features as that described above for normal and non-epileptic cortices (Fig. 3C). A slight neuronal loss was observed in the focus site of chronically seizing monkeys, especially in layers II and III (Fig. 3A). In the monkey with the most frequent seizure activity (RB-180), an increased number of glial cells were observed and the number of neurons was severely reduced.

#### *Quantitative analysis*

Multiple brain sections from each monkey were analyzed for the between-group comparisons using analysis of variance (ANOVA). The mean number of GAD-positive cells at each of 2 sites (focus and parafocus) was expressed as a percentage of the contralateral cortex. Since the mean number of these neuronal somata in the 2 contralateral sites of each monkey did not differ statistically ( $t = 0.274$ ,  $P = 0.786$ ), they were averaged

TABLE III

*Results of Nissl counts of neurons from 10 monkeys (number of somata/0.157 mm<sup>2</sup>)*

<i>Sample</i>	<i>Procedure</i>	<i>Right motor cortex</i>	<i>Left motor cortex</i>	<i>% change in left cortex</i>
RB-179	Normal	117	115	-1.7
RB-182	Sham	131	119	-9.2
RB-183	Sham	121.5	95	-21.8
RB-161	Pre-seizing	89.5	90	+0.6
RB-175	Pre-seizing	175.8	160	-9
RB-176	Pre-seizing	141.4	129	-8.8
RB-160	Acute	98.5	93.5	-5.1
RB-180	Chronic	131.5	51.5	-60.8
RB-184	Chronic	131	107.5	-17.9
RB-185	Chronic	127	113.5	-10.6

together to obtain a single value. The following statistical analysis of all 10 monkeys was based on these normalized values.

For the pre-seizing monkeys, the site adjacent to the alumina gel application showed a significant decrease in the numbers of GAD-positive neuronal somata relative to the contralateral cortex (Fig. 5). This reduction in the number of GABAergic neurons at the 'focus' for the pre-seizing monkeys ranged from 23 to 44% (Table II). These quantitative results supported the qualitative findings described above. In contrast, the number of GAD-positive cells in the parafocus of pre-seizing monkeys showed a smaller percentage difference (Fig. 5). These differences were of interest because normal and surgical control animals did not differ significantly in the numbers of GAD-positive neuronal somata at the different counting sites (Table II). When compared to the pre-seizing monkeys, the chronically seizing monkeys showed a larger reduction in the numbers of GAD-positive neuronal somata at both the focus and parafocus (Fig. 5). The data for chronically seizing monkeys (Table II) indicated a loss of GABAergic neurons at the focus and parafocus ranging from 42 to 61% and 15 to 26%, respectively.

A 1-way ANOVA showed significant differences between groups for both the focus ( $F(4, 38) = 19.4$ ,  $P < 0.0001$ ) and parafocus ( $F(4, 38) = 4.0$ ,  $P < 0.001$ ). The post hoc analyses with the Fisher Probable Least Significant Difference test showed



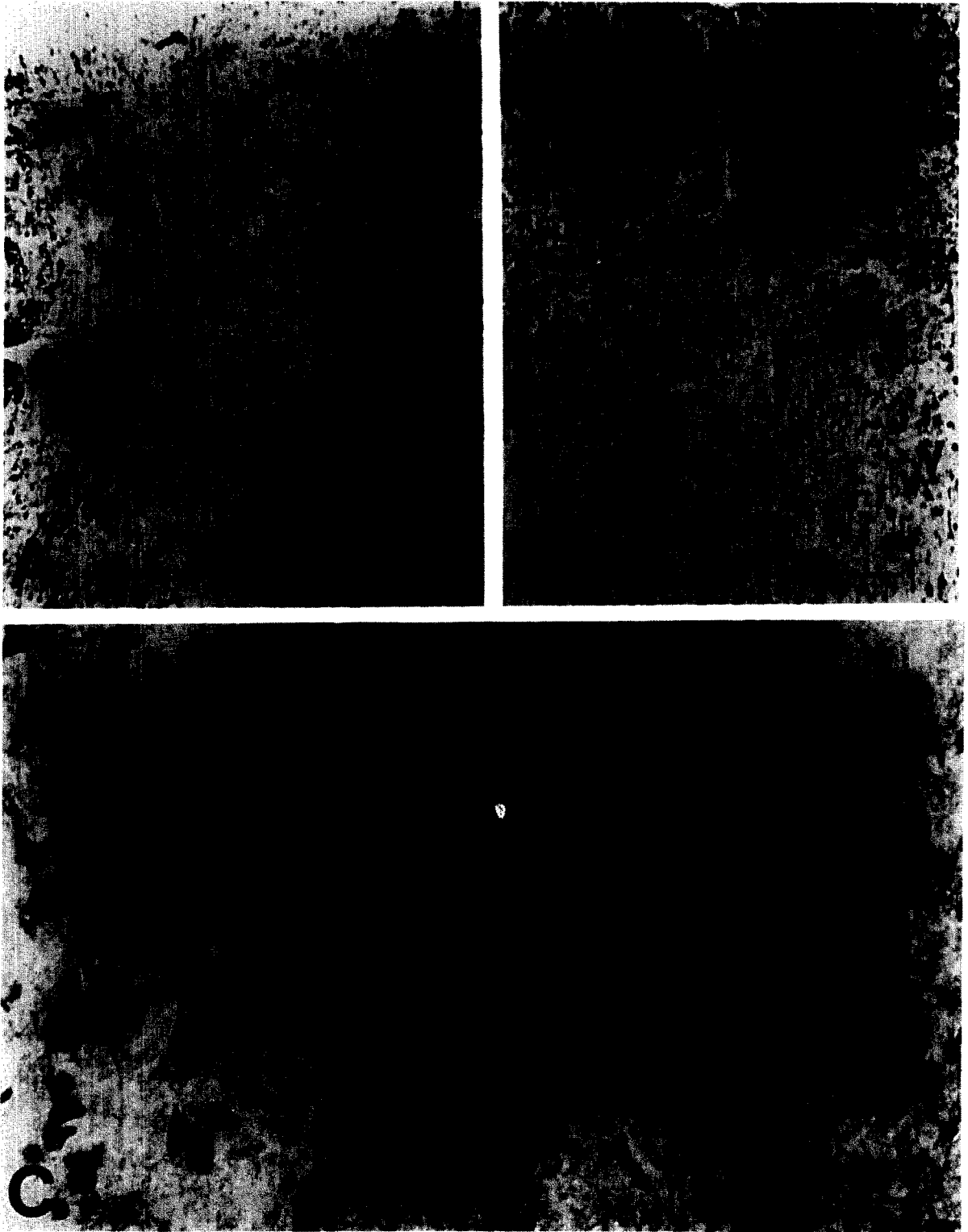


Fig. 3. Light photomicrographs of Nissl-stained sections of motor cortex from the chronically seizing monkey shown in Fig. 2. A shows the focus adjacent to the edge of the alumina gel granuloma (arrows). Layer I (I) is located at the top of this figure and the other cortical layers are indicated below it. B shows the contralateral motor cortex from the same monkey shown in A. The number and distribution of neurons in A and B are similar. However, layers II and III display a slight loss of neurons in A as compared to B. Bar = 100  $\mu$ m. C shows an enlargement of layer V from the focus in A. Pyramidal neurons (arrows) in many sizes show a normal morphology (cf., Fig. 4). Bar = 25  $\mu$ m.

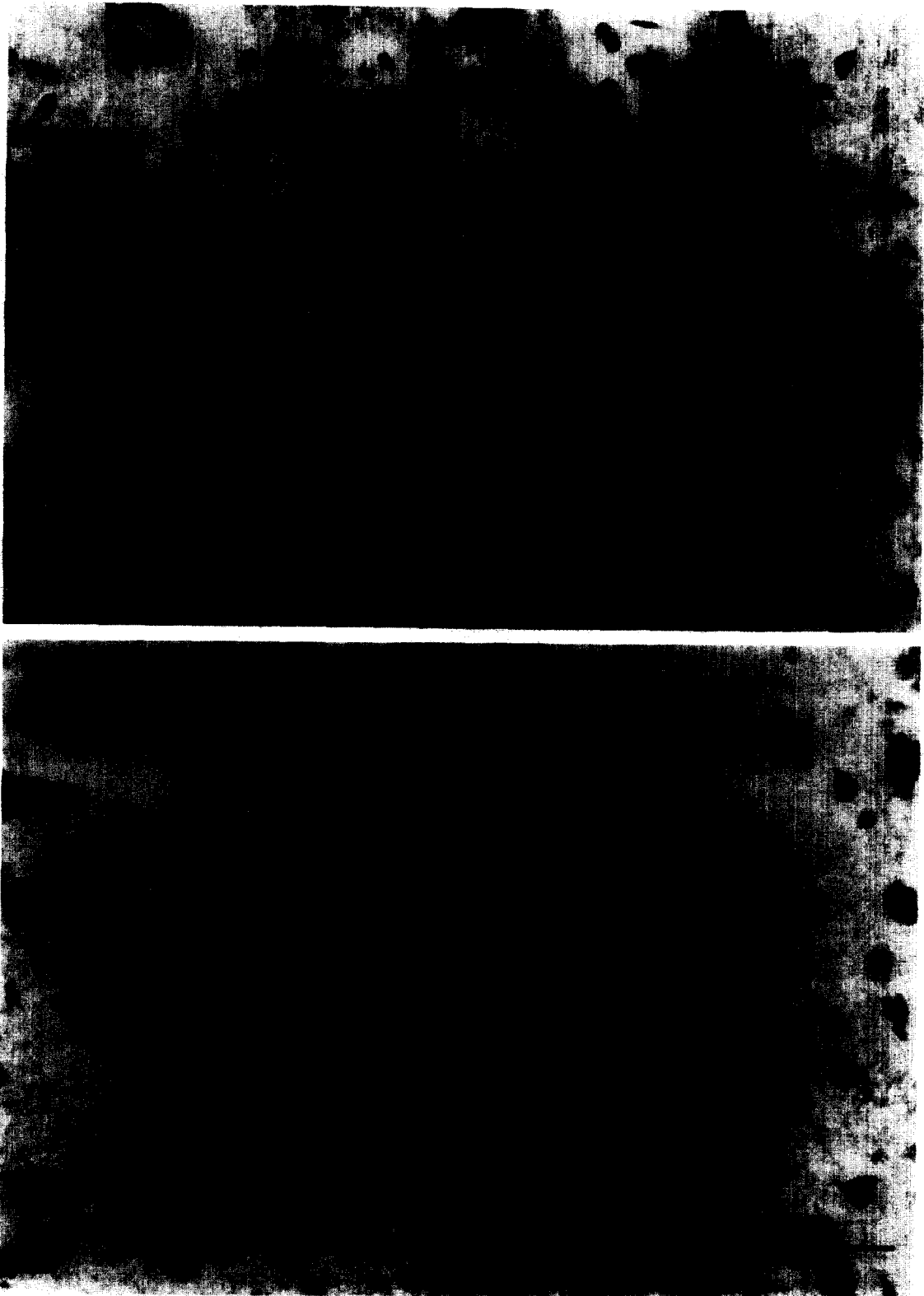


Fig. 4. Light photomicrographs of Nissl-stained sections of layer V of the motor cortex from the contralateral non-epileptic (A) and normal (B) cortices. Both show pyramidal cells (arrows) in various sizes. A was from the same chronically epileptic monkey (RB-184) as shown in Fig. 3. B was from RB-179, a normal monkey. Bar = 25  $\mu$ m.

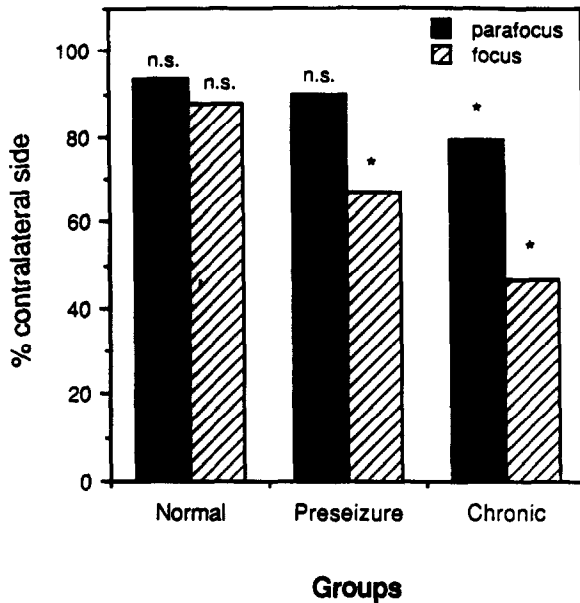


Fig. 5. Numbers of GAD-positive neurons as percentages of the non-epileptic, contralateral side of the monkey motor cortex. The chronic and pre-seizing groups were injected with alumina gel. The normal group included 2 sham-operated monkeys that were assessed for GABAergic neurons at 2 sites proximal and distal to the lesion which was not an epileptic focus. A normal monkey was also included in this category. \* Significant at  $P < 0.05$  level; n.s. = not significant.

that the reduction in the number of GABAergic neurons at the focus of the pre-seizing monkeys was significant ( $P < 0.05$ ), as compared to the sham-operated and normal monkeys. Also, the number of GABAergic neurons at the focus of pre-seizing monkeys was significantly less than that of chronically seizing monkeys ( $P < 0.05$ ). There was no significant reduction in the number of GABAergic neurons at the parafocus of pre-seizing monkeys. The chronically seizing monkeys also showed a significant loss of GABAergic neurons at the focus ( $P < 0.05$ ), and this was significant when compared to all other groups. In addition, this latter group of monkeys showed a significant loss of GABAergic neurons at the parafocus ( $P < 0.05$ ). None of the other groups of monkeys showed any significant loss of GABAergic neurons at the 2 sites.

The Nissl counts were made in both the right and left motor cortices. The left motor cortex was either the site for alumina gel application or the sham surgery. It is interesting to note that the loss

TABLE IV

Percentage loss of somata and terminals at alumina gel epileptic foci

	Terminal loss		Cell body loss	
	Pre-seizing	Chronic	Pre-seizing	Chronic
Ribak et al. <sup>19*</sup>	—	58–62%	—	—
Houser et al. <sup>8</sup>	14–22%	24–33%	—	—
Ribak et al. <sup>20</sup>	—	—	—	35–52%
Present study	—	—	23–44%	42–61%

\* Excluding the most chronic animal with status epilepticus.

of total neurons in the pre-seizing and acute monkeys at the focus site was similar to that found in the sham-operated monkeys (Table III). Statistical tests revealed no significant differences for these small reductions in cell number. In contrast, the 3 chronic monkeys displayed a significant loss of neurons that ranged from 11 to 61% ( $P < 0.05$ , Mann-Whitney U test). The monkey (RB-180) with the most severe loss of neurons was the same one that displayed the large increase in glial cell number (see above).

## DISCUSSION

The major finding of this study is the demonstration of a significant and selective loss of GABAergic somata at the focus site in the pre-seizing alumina gel-treated animals. The magnitude of the loss of GABAergic neurons was 23–44% as compared to the contralateral, non-epileptic cortex (Table IV). The magnitude of this significant loss contrasts with the non-significant percentage change of total neurons in the pre-seizing cortex that ranged from an increase of 0.6% to a loss of 9% (Table III). Therefore, the loss of GABAergic neurons at focal sites of alumina gel application in pre-seizing monkeys is selective for this neuronal type. In addition, these data suggest that GABAergic neuronal loss precedes the onset of clinical seizures, a finding that is consistent with the results of Houser et al.<sup>8</sup>, who showed a significant loss of GABAergic terminals in pre-seizing monkeys. Since this latter study showed degenerating terminals at the electron microscopic level, it is likely that the reduction in the number of GAD-immunoreactive somata reflects a degeneration of

these neurons and not simply a loss of immunocytochemically detectable levels of GAD in cortical GABAergic neurons as observed in the kindling model of epilepsy<sup>11</sup>. The loss of GABAergic neurons in pre-seizing monkeys is probably a result of the alumina gel implants because the surgical control animals (Table II) did not show a significant loss of GABAergic neurons. Therefore, the alumina gel may somehow affect the cortical vascular supply to cause ischemia which may be the cause of the selective destruction of GABAergic neurons as previously proposed<sup>18,22</sup>.

Another important finding of this study was that the chronically seizing monkeys displayed a significant loss of GABAergic neurons at both the focal and parafocal sites. A previous study showed a significant loss of GAD-positive cells at the focus<sup>20</sup>. However, the parafocal region in these same chronic monkeys did not show a significant loss<sup>20</sup>. This was probably due to the fact that those chronic animals were sacrificed 2–2.5 months after the alumina gel implant. In the present study, the chronic animals were sacrificed 3–6.5 months after the implant. These data add support to an old adage, that seizures beget more seizures. In other words, the longer the time interval between implantation of the alumina gel and the time of examination of brain tissue, the more seizures will have occurred and the more severe will be the degeneration of GABAergic neurons and terminals. Thus, the results of the present study show that alumina gel causes a significant loss of GABAergic neurons prior to seizures at the focus and that as seizures progress, more GABAergic neurons are lost at both the focus and parafocus (Table IV). However, it is unclear from these studies whether the seizures or the increased exposure to alumina gel is the cause of the loss of neurons in the parafocus of chronic monkeys.

The magnitude of the percentage loss of GABAergic neurons and terminals reveals some interesting relationships (Table IV). The study by Houser et al.<sup>8</sup> showed that a decrease of GABAergic terminals ranged from 24 to 33% in chronically seizing monkeys and from 14 to 22% in pre-seizing monkeys. The results of a previous study by Ribak et al.<sup>19</sup> showed a more severe deficit range of GABAergic terminals in chronic animals. This lat-

ter finding could have been due to the use of either chronically seizing monkeys with longer histories of seizures or thicker sections for counting than that used by Houser et al.<sup>8</sup>. The 2 studies that have analyzed the loss of GABAergic somata showed that the percentage loss of somata in chronic monkeys (Table IV) is more similar to the percentage loss of terminals in the study by Ribak et al.<sup>19</sup>. Together, these studies demonstrate a significant loss of GABAergic cells and terminals in the focus and parafocus of chronically seizing monkeys as well as at the site of alumina gel application in pre-seizing monkeys. They also indicate that the magnitude of these losses increases as the seizures develop. Thus, the GABAergic somata and terminals are decreased in number prior to the onset of clinical seizures and this decrease becomes more severe after seizure development. These findings add further support to the hypothesis that GABA loss plays a causal role in focal epilepsy.

Two of our previous studies have provided data to determine which GABAergic cell types are affected most at chronic alumina gel epileptic foci. One study<sup>18</sup> showed that axosomatic synapses formed by the basket cell endings were severely lost. A subsequent study<sup>17</sup> reported a significant loss of axon initial segment synapses formed by GABAergic chandelier cells. It is interesting to note that both chandelier and basket cell types do not co-localize neuropeptides because the 3 peptides (somatostatin, cholecystokinin, neuropeptide Y) that are known to be co-localized to GABAergic neurons are found in other cortical cell types<sup>7,23</sup>. Therefore, it is likely that most GABAergic neurons lost at epileptic foci are using only GABA as a neurotransmitter.

This study provides additional evidence to show that the GABAergic neuronal system is disrupted in some varieties of epilepsy. Biochemical studies of similarly treated monkeys have confirmed the loss of GAD activity in epileptic foci<sup>1</sup>. Similar losses have been shown for the GABA receptors<sup>1</sup>. Secondly, axosomatic symmetric synapses<sup>16</sup>, the type formed by terminals of GABAergic basket cells, have been shown to be reduced in number at experimental epileptic foci<sup>4,18,21</sup>, and terminals that form such symmetric synapses were shown to degenerate in pre-seizing monkeys<sup>8</sup>. Taken to-

gether with data from the present study that indicate that a selective loss of GABAergic neuronal somata occurs in pre-seizing monkeys at the site of the alumina gel granuloma, it is evident that damage to the cortical GABAergic inhibitory system is a common finding for experimental models of post-traumatic focal epilepsy.

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